

ABSTRACT

A method for balancing multiplexed PCR methods is provided. In the method, two or more sequential temporal PCR stages are used to effectively separate two or more PCR reactions in a single tube as an alternative to primer limiting to modulate the relative rate of production of a first amplicon by a first primer set and a second amplicon by a second primer set during the first and second amplification stages. Also provided are rapid RT-PCR methods that find particular use in intraoperative diagnoses and prognoses, for instance in diagnosing malignant esophageal adenocarcinoma by determining expression levels of carcinoembryonic antigen (CEA) in sentinel lymph nodes.